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Review article

Framework for risk assessment of PFAS utilizing experimental studies and in-silico models

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ABSTRACT

Perfluoroalkyl substances (PFAS), especially PFOS and PFOA, are two widely used synthetic chemicals that can impact human health based on evidence from animal and epidemiologic studies. In this paper, we have reviewed and summarized the influence of PFAS exposure on health, pointing the quality of evidence, and applied translational techniques to integrate evidence for PFAS policy making. This is the first review where highly referred articles on PFAS used for policymaking by several regulatory agencies were collected and evaluated based on the review guidelines developed by the US National Toxicology Program's Office of Health Assessment and Translation (OHAT) review guidelines. Several limitations were observed, including co-exposure to multiple chemicals and limited measurement of primary and secondary outcomes related to specific toxicity. However, data from all the studies provided a moderate to strong level of confidence for link between PFAS exposure and different adverse outcomes. Secondly, for translating the risk to humans, an in-silico model and scaling approach was utilized. Physiologically based pharmacokinetic model (PBPK) was used to calculate the human equivalent dose (HED) from two widely accepted studies and compared with tolerable daily intakes (TDIs) established by various regulatory agencies. Inter-species dose extrapolation was done to compare with human the relevance of dosing scenarios used in animals. Overall, a framework for translation of risk was proposed based on the conclusions of this review with the goal of improving policymaking. The current paper can improve the methodological protocols for PFAS experimental studies and encourage the utilization of in-silico models for translating risk.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic organic substances with perfluoroalkyl moieties that are highly resistant to environmental and metabolic degradation. Most PFAS are either non-degradable or transform into other stable PFAS metabolites (Pelch et al., 2019). Under EU chemicals regulation, they are classified as very persistent substances (Schrenk et al., 2020). PFAS are widely used in consumer products such as furniture, household cleaners, and clothing. Due to their resistance to environmental and metabolic degradation, many of these chemicals are increasingly found in the environment, as well as the human body, all over the globe (Wang et al., 2017). Among all PFAS, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been the most widely studied, due to their toxic effects on living beings and their common use in consumer and industrial applications (Chang et al., 2016). Regulatory agencies, for example the European Food Safety Authority (EFSA), the United States Environmental Protection Agency (USEPA), and the Agency for Toxic Substances and Registry (ATSDR), consider PFAS a public health concern, having established the tolerable daily intake (TDI) or reference dose (RfD) based on developmental toxicity and other toxic endpoints (Schrenk et al., 2020; USEPA, 2016; EFSA., European Food Safety Authority, 2012). Our current understanding about effects of PFAS is based on studies focused on a relatively small number of compounds. Little is known about the properties and behaviors of most PFAS's, either as

; RPF, Relative Potency Factor.

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Abbreviations

ADME	Absorption, distribution, metabolism, and excretion
ADONA	4,8-dioxa-3H-perfluorononanoate
ALT	Alanine aminotransferase
AOP	Adverse Outcome Pathway
ATSDR	Agency for toxic substances and Registry
AUC	Area under the curve
BMDL	Benchmark dose (lower confidence limit)
Cmax	Peak serum concentration
CRP	C-reactive protein
CONTAM	Contaminants in the Food Chain
DDEF	Data derived extrapolation factors
DDEs	Dichlorodiphenyldichloroethylenes
EFSA	European food safety authority
FSANZ	Food Standards Australia New Zealand
GFR	Glomerular filtration rate
GSH	Glutathione
HC	Health Canada
HDL	High-density lipoprotein
HED	Human equivalent dose
IATA	Integrated Approaches to Testing and Assessment
IQR	Interquartile range
LC-MS	Liquid chromatography-mass spectrometry
LOAEL	Lowest observed adverse effect level
MAC	Maximum acceptable concentration
MCMC	Markov Chain Monte Carlo
MRL	Minimal Risk Level
NAM	New approach methodologies
NHANES	National Health and Nutrition Examination Survey
NOAEC	Comparable no observed adverse effect concentration
NOAEL	No observed adverse effect level
OHAT	Office of Health Assessment and Translation
PBPK	Physiologically based pharmacokinetic modelling
PCBs	Polychlorinated Biphenyls
PFAS	Per- and polyfluoroalkyl substances
PFBS	Perfluorobutanesulfonic acid
PFDA	Perfluorodecanoic acid
PFDoDA	Perfluorododecanoic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexanesulfonic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
QSAR	Quantitative structure-activity relationship
RfD	Reference dose
TDI	Tolerable daily intake
TWI	Total weekly intake
USEPA	United States Environment Protection Agency

individual chemicals, or as the much more commonly present complex mixtures. Diversity among various PFAS based on structures, properties, accumulation potential and toxicity, leads to frequent arguments about whether all PFAS can be treated as a single class of chemicals (Bowman, 2015). However, despite their diversity, PFAS share one common structural feature that makes them highly problematic - the presence of perfluoroalkyl moieties, resulting in their shared resistance to environmental and metabolic degradation (Cousins et al., 2020; Wang et al., 2017). It has been scientifically accepted that the diversity of these substances, in terms of properties, behavior, hazards, and risks, is a significant criterion for risk classification. Categorizing a large group of chemicals based upon single structural components has the potential to be overgeneralized and debatable.

From 2000 until today, a number of epidemiological, animal, and in-vitro studies have found links between PFAS and health effects. Developmental toxicity (Gaballah et al., 2020; Lau, 2019), carcinogenicity (Singh and Hsieh, 2021), weight gain (Mitro et al., 2020; Ashley-Martin et al., 2016), hormonal imbalance (Ashley-Martin et al., 2016; Tsai et al., 2015), and immunity-related effects (Abraham et al., 2020), are some of the adverse effects reported in the literature (Sunderland et al., 2019). It has been noted that some studies contain controversies, as well as lack of scientific and statistical rigor of evidence to reach stated conclusions. The most common of these include statistical bias in interpreting results, bias in the convincing calculation of half-life, which includes occupational or real-time exposure, and uncertainty of in-silico models in predicting daily intake or kinetics of such chemicals (Chang et al., 2016).

Human health effects for some PFAS exposures have also been reported from animal models. However, a longer half-life, no metabolism, and high resorption of these chemicals make extrapolation from animal models to humans complicated. For PFOA and PFOS, half-life varies among rats (PFOA: 0.08–5.68 days, PFOS: 14.5–71.13 days), mice (PFOA: NR, PFOS: 30.45–42.81 days), cynomolgus monkeys (PFOA: 19.5–32.6 days, PFOS: 110–132 days) and humans (2–4 years, 5 years) (Schrenk et al., 2020; Knutsen et al., 2018). Considerable differences in biological half-lives among different species, as well as the proper accounting for this information in the extrapolation of dose and risk for humans, are still crucial issues. An additional major controversy lies in the varying renal clearance and resorption capability of these chemicals among different age groups (Deepika et al., 2021). Variations in the glomerular filtration capacity, protein binding, and disposition of the chemicals may lead to varied toxicokinetic and calls for differences in TDI among sensitive age groups.

Taking into account all of the above, the purpose of this study was to provide a summary of the experimental findings including the strengths and limitations in the methodologies and results of the research studies. These studies were identified by an independent group of experts for the Center for Truth in Science (CTS). The Center selected these studies because they were among the most widely cited, or most influential studies used by policymakers, PFAS manufacturers, environmental groups, plaintiffs, attorneys, social media influencers, and the news media to express views about the potentially harmful effects of these compounds, how they should be regulated, and whether individuals and organizations damaged by exposure to these compounds should be compensated. Various studies have been used to estimate a particular “safe” dose assessment by governmental agencies. Some investigations have provided vital information to enable credible extrapolation between experimental animals and humans. Other studies may have produced inconsistent findings or expressed dissenting scientific claims. For example, minimal risk level (MRL) derived by ATSDR is based on the study of neurodevelopmental effects conducted by Koskela et al. (2016) in the offspring of mice that were fed a diet containing PFOA. Similarly, the human equivalent dose (HED) and the lowest observed adverse effect level (LOAEL) for PFAS were estimated by the Agency for Toxic Substances and Disease Registry (ATSDR) with the data reported by the Luebker et al. (2005). The current analysis is aimed at determining the internal validity of each individual study based on accepted scientific standards for best practices. For this, we critically reviewed the selected scientific publications having multiple endpoints, evaluated and rated them based on confidence, and further applied the previously validated and published in-silico tool (PBPK) to assess the criterion of risk assessment for PFAS with the goal of improving policymaking (Fig. 1). Moreover, based on immunotoxicity, the predicted daily dose was compared with those established TDI/RfD by EFSA and USEPA. In

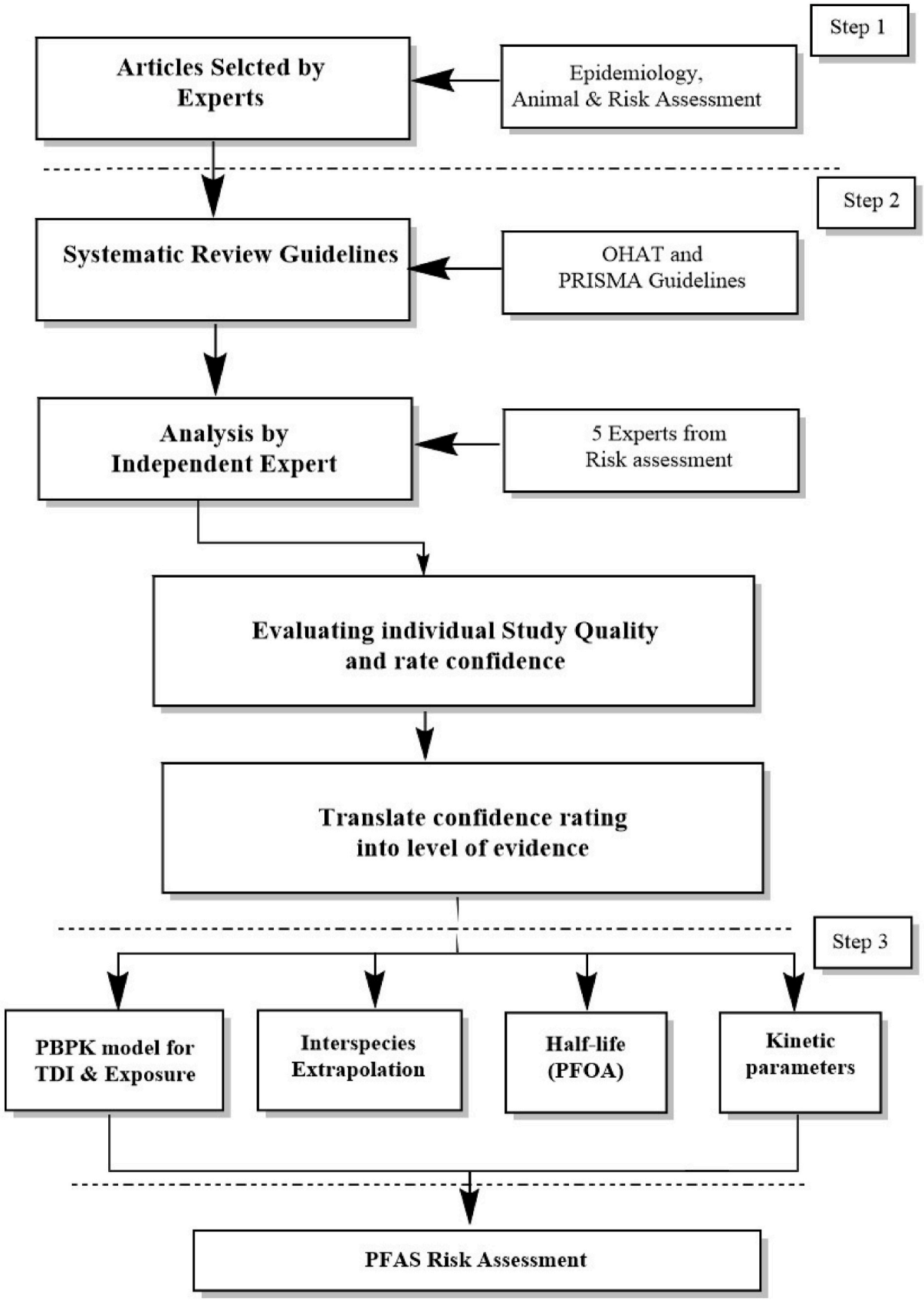

Fig. 1. Framework followed for evaluating the quality of evidence in the selected studies. OHAT and Prisma guideline were used for evaluating strength and weakness in different studies followed by in-silico models and other kinetic assessment for human health.

addition, scaling approaches for reducing inter-species differences were analyzed (Fig. 1). Finally, an integrated framework for risk translation is proposed.

2. Analysis of research articles using the OHAT guideline

To evaluate the quality of evidence and research protocols of the selected articles regarding PFAS, a risk-of-bias tool which was developed using OHAT guidelines was employed. It outlines a parallel approach to assess risk of bias from human, animal, and in vitro studies in order to facilitate consideration of the risk of bias across evidence streams (NTP, 2015). This evaluation process was developed by consulting experts from the Cochrane Collaboration, the Grading of Recommendations Assessment, Development and Evaluation (GRADE) working group, the Collaborative Approach to Meta-Analysis and Review of Animal Experimental Studies (CAMARADES), the Systematic Review Center for Laboratory animal Experimentation (SYRCLE), Navigation guide, and other experts, and in accordance with PRISMA guidelines.

Different types of scientific studies focused on analytical investigations, epidemiology, and kinetic assessments were included. Justification for why these specific studies were selected for PFAS are summarized in Table 1. Five of the selected articles focused on animal studies, primarily rats and mice, that evaluates chronic and acute toxicity with multiple endpoints. The four epidemiological studies were based on immunotoxicity, drinking water exposure, and half-life in humans (shown in Table 1). In total, the key health outcomes named in these articles included: immunotoxicity, carcinogenicity, bone toxicity, in-utero toxicity, two generational exposure, organ toxicity, and chronic toxicity. For the independent review by experts, a review template was developed consisting of questions related to methodology protocols, randomization procedures, statistical evaluation, publication biases, outcomes measured, consistency, and confounding (Appendix 2). The overall rating of the reviewed articles based on the aforementioned aspects were as follows: first, the risk of bias was reported; secondly, the level of evidence and confidence ratings were analyzed (supplementary file, Tables 3 and 4). All individual scoring was integrated in order to create a rating of confidence in the risk assessment for PFAS chemicals.

Table 1
Summary Table of the human and animal studies selected by CTS for this review.

Brief Description about why the studies were selected	Reference
Internal exposures to PFAS matched with biological markers and vaccine responses; the principal study used by the European Food Safety Authority (EFSA) in its recent risk assessment.	Abraham et al. (2020)
Investigation of community exposures to PFOA and serum concentrations.	Emmett et al. (2006)
Serum vaccine antibody concentrations monitored in children exposed to PFAS chemicals highlighted by the European Food Safety Authority (EFSA) in its recent risk assessment.	Grandjean et al. (2012)
Study on half-life of several PFAS compounds in the serum of retired production workers that is cited by all authorities in their assessment work.	Olsen et al. (2007)
Chronic study in rats showing Leydig cell tumors; this is the principal study used by Health Canada in its 2018 assessment.	Butenhoff et al. (2012)
Short term gestational exposure in mice with implications for long term effects; this is the principal study used by the Agency for Toxic Substances and Disease Registry (ATSDR) in its determination of minimum risk level.	Koskela et al. (2016)
Effects of PFOA on pregnancy in mice; this is the principal study used by U.S. Environmental Protection Agency to estimate its health advisory of 70 ppt.	Lau et al. (2006)
Liver changes in rats associated with PFOA exposure; this is the principal studies used by Health Canada in its 2018 assessment.	(Perkins et al., 2004) and (Luebker et al., 2005)

3. Quality of evidence and limitation of studies

Based on the quality and the limitations we identified (Tables 3 and 4 in appendix), the level of evidence for PFAS health effects in the articles were listed in Tables 1 and 2 (supplementary file appendix 1), was integrated. Also, several issues related to outcomes and methodology from the studies were identified by our independent experts mentioned below.

The first one concerns the effects of co-exposures on the human body. For instance, a similar adverse effect can be the result of multiple chemicals, especially in the case of epidemiological studies that are difficult to control. There may already be existing exposures affecting a particular toxicity endpoint, or leading to toxicokinetic interference (e.g., competing for the elimination of the chemical). Especially in the case of PFAS, the toxicity of mixed PFAS's and their interactions with each other and other classes of chemicals is currently a significant knowledge gap in the area of risk assessment (Ojo et al., 2021). Inter-individual variability should also be considered especially for end-points like immunotoxicity. Two epidemiological studies (Abraham et al., 2020; Grandjean et al., 2012) used standard immune antibody biomarkers for their immunity endpoints, but there are certain other standard biomarkers, such as leukocyte count and c-reactive protein (CRP) that need to be take into account. Although, Abraham et al. (2020) did consider the leukocyte count and other clinical biomarkers, the study by Grandjean et al. (2012) assessed only antibody levels. Another important point is the variation in antibody level with time. A single sample point and a small sample size as reported by Abraham et al. (2020) may not be sufficient to give definitive causative information. In one of the epidemiological studies, the participants were aware of the study and the protocols. In this case, the questionnaire given to participants might have been biased, especially if the questionnaire involved self-reported outcomes. In such cases, adverse outcomes reported by the participant may or may not hold true, if they know that they are being exposed to PFOA through drinking water (Emmett et al., 2006). There was no cross-check of the administered questionnaire mentioned by the author in the Emmett's article, being the results still used for statistical analysis (Emmett et al., 2006). Some of the included articles were published by manufacturers of PFAS. However, in each of these cases, one or more authors were from outside the sponsoring organization, indicating the chance for some more rigorous scientific review and minimization of possible bias. Considering the fact that the funding sources are manufacturers, there could be a probability of bias. This was included in the protocol, but it was not assumed initially. The methodology reported outcomes and data quality were evaluated to further report any suspected bias.

There were many strengths identified in the included studies, which increases the confidence for conducting risk assessment. In the animal studies, the primary and secondary outcomes were measured for specific endpoints. For example, in the carcinogenicity study for PFOS exposure in rats, a necropsy was done to measure weight, and a histopathology study was conducted on major organs. In addition, clinical observation, body weight, food consumption, hematology, urine analysis, etc. were conducted to determine secondary outcomes (Butenhoff et al., 2012). Complete outcome assessment improved the confidence level and helped to reduce bias. In most studies on rodents, randomization was done to select control and exposed groups, and in some cases, an automated randomization procedure was used. All exclusion criteria, including the death of animals, and results of pathology were reported by the various authors (Butenhoff et al., 2012; Luebker et al., 2005). Feed consumption and serum levels were measured to exclude any inconsistencies. In the two epidemiological studies, PCBs, which have the potential to affect immunological results, were measured by both authors to reduce statistical bias (Abraham et al., 2020; Grandjean et al., 2012). Heavy metals, such as cadmium, lead, mercury, and DDEs were measured by Abraham et al. (2020), including all clinical outcomes. In most studies, standard statistical analyses were followed-up with multiple tests,

including p values and other required methods. In most cases, significant and non-significant results were reported by authors for both human and animal studies (Abraham et al., 2020; Koskela et al., 2016; Butenhoff et al., 2012; Grandjean et al., 2012; Lau et al., 2006). In some animal studies, a large sample size was used to reduce the variability (Butenhoff et al., 2012; Lau et al., 2006; Luebker et al., 2005; Perkins et al., 2004). In the two epidemiological studies, repeated sampling of blood/serum was sometimes conducted to avoid measurement bias (Grandjean et al., 2012; Olsen et al., 2007). Most analyses of the chemicals were done by LC-MS (liquid chromatography-mass spectrometry), a standard method used for analysis. In many cases, the analyses of samples were repeated to increase the accuracy of the data.

4. Risk of bias and confidence levels in individual studies

This section focuses on the risk of bias detected in methodological protocol reported, level of evidence, shortcomings, and the significant findings from each individual study.

4.1. Animal studies

A 2-year dietary exposure study (PFOS) by Butenhoff et al. (2012) in which different concentrations were used in male and female Sprague Dawley rats (n = 720), was quite extensive. In males, total cholesterol levels decreased, while total urea levels increased with the 5 and 20 μg/g (ppm) diet. Hepatocellular carcinoma in the females and increased hepatocellular adenoma in both males (p = 0.046) and females (p = 0.039), was detected for the 20-ppm exposure group. In the methodology protocol and statistical analyses, a low risk of bias was detected. Randomization of the rats was done based on an automated blocking procedure. Considering the methodology and outcomes reported, the level of evidence was high for PFOS contributing to adverse effects in rats, particularly in the liver, but significant adverse effects were observed specifically in the 20-ppm group.

Koskela et al. (2016) evaluated the effects of PFOA (0.3 mg/kg/day) in-utero and during lactational exposure on the bones of female offspring of mice at 15 (n = 5) and 17 (n = 5) months. The methodology protocol had a low risk of bias based on specific inclusion and exclusion criteria. However, one of the major concerns with this study was the dose selection procedure and use of single dosing. Additionally, detail about randomization of both the control and exposed groups were not articulated clearly, except for the fact that one or two female offspring from the same litter were randomly selected for inclusion into the exposed group. Also, for PFOA analysis in bones, the samples were pooled, and aggregate data were presented. An inconsistency was observed in one figure that was reported in the osteoblast viability study, where the authors reported that an increase in calcium peaked at 1 and 10 μm dose and further decreased at 100 and 200 μm, compared to controls. Overall, a rating of moderate to high level of evidence was given to this study, due to inconsistencies in some of the reported results as well as the use of aggregated data.

In the study by Lau et al. (2006), pregnant CD-1 mice and Sprague-Dawley rats were exposed to different PFOA concentrations in order to evaluate developmental toxicity. A low risk of bias was detected in methodology, as well as in statistical evaluation, as the mice were exposed to different concentrations and randomization was used to select animals. Also, exclusions were clearly reported with rationale and details. For instance, neonates in the 10 and 20 mg/kg dosing groups did not survive, while in the 40 mg/kg dosing group, no births were reported. A good sample size of rats was included in each group to reduce variability and uncertainty. This study demonstrates a high level of evidence.

PFOA exposure was related to prenatal and postnatal toxicity in mice and sex-related differences in reproductive maturity between males and females. Luebker et al. (2005) conducted a two-generation study in rats to evaluate the PFOS effects on reproduction, pregnancy, and offspring development. Different doses (0, 0.1, 0.4, 1.6, and 3.2 mg/kg) were administered before six weeks of mating and continued through gestation and lactation. A low risk of bias was detected in the methodology and statistical analyses. However, all samples went to a 3M laboratory for analysis, and were found to be accurate to ±30%. Overall, it was concluded that there was a high level of evidence that PFOS could contribute to prenatal and postnatal toxicity in mice.

A thirteen-week dietary toxicity study was conducted in male rats in order to evaluate liver effects, hormonal changes, and effects on other target organs (Perkins et al., 2004). A low risk of bias was detected in methodology and statistical evaluation. The strengths of this study were a low statistical bias due to the use of a large number of animals and a computerized randomization process with multiple dosing ranging up to 100 ppm. There was a high level of evidence that the liver is a target organ of PFOA toxicity. At 100 ppm, liver weight change, enzymatic activity, and hepatocellular hypertrophy were observed with a LOAEL of 10 ppm.

4.2. Epidemiology studies

Abraham et al. (2020) reported that exposure to PFAS especially PFOA, in Germany had negative associations with serum levels, as well as negative immune response against three vaccine antibodies with high consistency and comparable no observed adverse effect concentrations (NOAEC). Significant associations were detected for PFOA exposure and Haemophilus influenza type B (Hib) (r = 0.32), tetanus (r = 0.25), and diphtheria (r = 0.23) with NOAEC of −86, −54 and −53%. Mean serum levels (ng/ml) detected in 101 healthy, one-year old, formula-fed children and breastfed children for PFOA were 3.8 ± 1 and 16.8 ± 6.6 respectively, for PFOS 6.8 ± 3.4 and 15.2 ± 6.9 respectively. Overall, a low risk of bias was detected based on the methodology. The data had high variability but presented statistically significant results. For instance, the ranges of IgG (Hib and diphtheria) and IgG1 (tetanus) antibody levels, (Hib: 0.026–100 mg/L, Diphtheria: 0.009–4.45 IU/mL, tetanus: 0.286–191 mg/L), were quite broad. An additional point to be noted is that multivariate analysis with few exclusions and the existence of confounders in a small sample size may lead to statistical bias. However, statistical bias with respect to the variables, co-exposure, and protocol deviation was found to be quite low. In addition, based on outcomes (primary effects such as antibody response and clinical parameters) and other factors (dose-response, magnitude of effect etc.), overall confidence was found to be high. Based on the methodology and results reported in this article, there is convincing evidence that PFOA exposure is associated with a reduced level of vaccine antibody response in children.

The association of PFOA exposure from air and water and human serum concentration levels was investigated to identify potential exposure sources for people living near production facilities in the U.S. (Emmett et al., 2006). Residential Drinking water was found to be a contamination source responsible for blood serum levels with little contribution from air exposure. There was a low risk of bias found in the methodology and statistical evaluation. However, a significant risk of bias identified in the study was the exposure characterization of a diverse population group (2–60 years) over a large exposure period. Serum levels were found to be considerably higher in the age group >60 years (approx. IQR 217–874 ng/ml) followed by the 2-5-year-old age group (approx. IQR 385–780 ng/ml) and the 51-60-year-old age group (approx. IQR 134–576 ng/ml). The use of an air dispersion model to identify areas with higher PFOA exposure from the air was one of the strong points of this study, avoiding the risk of bias from other potential sources. A high level of evidence of the association of PFOA exposure from drinking water and blood serum levels was found, but this evidence perhaps not present the complete picture, since exposure might be from additional sources.

Grandjean et al. (2012) conducted a prospective birth cohort study in the Faroe Islands, which included approximately 656 singleton births

recruited from 1997 to 2000, and 587 who were followed up in 2008. Antibody concentration against diphtheria and tetanus was measured for children aged 5–7 years with significant results. A low risk of bias was detected in the protocol and statistical analysis. Parameters including age, sex, and vaccine schedule were considered for this study. There was still potential for a high risk of bias due to possible confounders such as co-exposure and other population confounders, which could have affected the results. The study was adjusted for some known confounders such as PCBs and duration of breastfeeding. However, it didn't consider others including co-exposure to other PFAS (there is limited ability to differentiate effects of PFOA or PFOS from other PFAS). The selected cohort population in the Faroe Islands had known exposure to PCBs, which has been shown to suppress the antibody response. However, the analysis accounting for adjustment of PCBs showed no effects on the principal outcome. Nevertheless, exposure to PCBs was monitored along with PFAS. The percentage difference in antibody concentration at 5 years of age prebooster, postbooster, and 7 years of age for year 5 (prebooster: −30.9 to 10.9, postbooster: −45.5 to 6.1 and 7 years of age (−44.3 to 4.2) in case of tetanus and for diphtheria for 5 (prebooster: −34.9 to 8.3, postbooster: −31.5 to 4.3 and 7 years of age (−45.8 to 3.3) was quite wide for PFOS. For PFOA, the difference was also quite significant. Moderate to high evidence was found for available data on PFAS, and especially on immunity, for PFOS and PFOA.

The elimination half-life was calculated for PFOS, PFHS, and PFOA from human serum in retired fluorochemical production workers based on first-order elimination (Olsen et al., 2007). There was a low risk of bias detected in this study. Calculating half-life without random sampling of participants and strict inclusion criterion were seen as a bias. Whether the 26 retirees were suffering from any disease or ailment was neither checked, nor specified, which can lead to altered elimination of these chemicals, especially when all the participants were older than 50 years of age, and likely had previous exposure. Another important question was whether the exposure source was constant or varying. The only information available from the article was that the sampled population was not working at the APFO production facility any longer. However, there could be other exposure sources such as living in a polluted area, or drinking PFOA polluted water. For calculating the half-life of these chemicals, first-order kinetics was used, taking into account that the exposure might have decreased from the initial to the second sampling. However, this decrease could have been due to reduced exposure and the ban on PFAS chemicals after the year 2000. Moderate risk of bias was detected in methodology and half-life calculation. No confounders or modifying variables were considered during calculations, especially co-exposures or disease pathology.

5. Risk assessment for PFAS chemicals to improve policymaking

Based on the above, there were certain questions, which emerged related to translation of risk: 1) calculating exposure level of PFOS/PFOA for plasma/serum concentration detected during immunotoxicity in children of different ages and its comparison with TDI/RfD; and 2) the dosing used for animal studies and its relevance for human risk assessment. The second part of the review focused on all the above questions for finding the gaps and improving risk assessment. A pharmacokinetic assessment was done utilizing dosimetry modeling for reconstructed exposure. Published physiologically based pharmacokinetic (PBPK) models for PFOS and PFOA were used, which simulated the Markov Chain Monte Carlo (MCMC) approach for calculating reconstructed exposure taking into account parametric uncertainty (Deepika et al., 2021; Rovira et al., 2019; Fàbrega et al., 2014). With the help of PBPK models, expected daily exposure was calculated using reverse dosimetry from the observed plasma/serum level concentration reported by Abraham et al. (2020) and Grandjean et al. (2012). Results were then compared with TDI/RfD set by EFSA and USEPA. Further, inter-species extrapolation was done to compare the dosing scenario used in the animal studies and its relevance was evaluated, with respect to the daily exposure set by regulatory agencies.

5.1. PBPK modelling and extrapolation

In 2020, total weekly intake (TWI) was set by EFSA to 4.4 ng/kg BW/day, based on a reported decreased immune response to vaccination. Two widely cited articles, Abraham et al. (2020) and Grandjean et al. (2012), concluding that there is a decreased immune response in infants and children after PFAS exposure, were evaluated. Previously validated and published PBPK Models by our group for PFOS and PFOA (Deepika et al., 2021; Rovira et al., 2019; Fàbrega et al., 2014) were used to calculate the reconstructed exposure and to compare it with the TDI by EFSA and USEPA (appendix 3, Tables 5 and 6). In relation to the EFSA 2020 limit (the concentration at which immunotoxic effects are observed) the TDI is 100–1000 times lower for the minimum concentration simulated for PFOS and PFOA. Compared to the USEPA guidance value, where TDI or RfD is 0.02 μg/kg BW/day, the TDI is only 1–10 times lower than the concentration at which toxic effects were observed in children for PFOS and PFOA (Figs. 2 and 3). For USEPA, considering the safety and uncertainty factors, it may be equivalent to a toxic dose.

5.2. Inter-species dose extrapolation

Extrapolation of doses between species, from animals to humans, and their relevance in policy making is an important issue for toxicity assessment. Human equivalent dose (HED) scaling approaches, including allometric scaling, ontogeny scaling, pharmacokinetically guided scaling, etc., are some of the methods used. However, the most acceptable scaling method used today is allometrically based on body surface area, due to limited data availability for supporting other extrapolation methods (Nair and Jacob, 2016). Five articles which were reviewed above related to animal studies (Koskela et al., 2016; Butenhoff et al., 2012; Lau et al., 2006; Luebker et al., 2005; Perkins et al., 2004) were used to evaluate dose scaling (Table 7, appendix 3). The United States' Food and Drug Administration (USFDA) formulated an interspecies dose conversion table, which was used for calculating HEDs, by multiplying or dividing NOAELs (no observed adverse effect levels) by a conversion factor. For calculating the dose, a safety factor of 10 was taken into account based on variability in extrapolation from animals to humans. There can be many reasons for variability in both species; unanticipated toxicity in human which may not be observed in animals, variation in metabolism and elimination, or increased sensitivity towards toxicity in humans compared to animals. Table 2 shows references for animal to human scaling based on BSA. A similar approach was used to calculate HED based on animal studies of PFAS.

$$HED = \frac{Animal\ dose\ in\ mg}{kg} * \left(\frac{Animal\ weight\ in\ kg}{Human\ Weight\ in\ kg}\right)^{0.33} \quad (1)$$

When comparing the human equivalent dose to the TWI set by the EFSA after including a safety factor of 10, the dosing used in animals is approximately six to fifty-two thousand times higher than the lower dose used in the studies (Fig. 1, Supplementary file). However, compared to the TDI set by USEPA, which is relatively high compared to that of the EFSA, the dosing used was between forty and four hundred times higher (Fig. 1, Supplementary file). The dosing used in the animal studies is quite appropriate as per limit set by USEPA to find toxic effects and to understand risk assessment in humans. But compared to the EFSA limit, animal dosing is very high, raising a question as to whether these animal studies can be considered valid for human risk assessment. Nevertheless, critical questions remain. These include the following: Is the dose scaling with respect to body surface area relevant when the half-life of chemicals such as PFOA varies from 28 days to 1.2–8.5 years (Schrenk et al., 2020) in humans? Should we consider the pharmacokinetic phase from first order to zero order in relation to clearance and consideration of varied volume of distribution? Should species differences in glomerular filtration and the number of nephrons should be considered in allometric

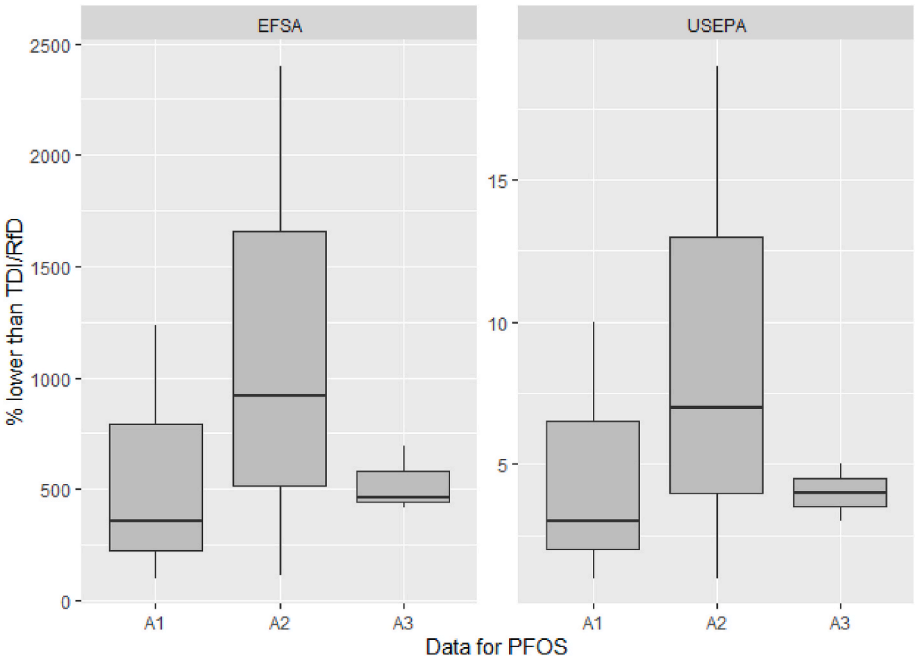

Fig. 2. Comparison with the TDI or RfD set by regulatory bodies for PFOS. A1 and A2 represent the daily exposure simulated of 1-year-old children formula-fed (n = 21) and breast-fed (n = 80), respectively (Abraham et al., 2020). A3 represents the daily exposure simulated by PBPK for 5-year-old children (Grandjean et al., 2012).

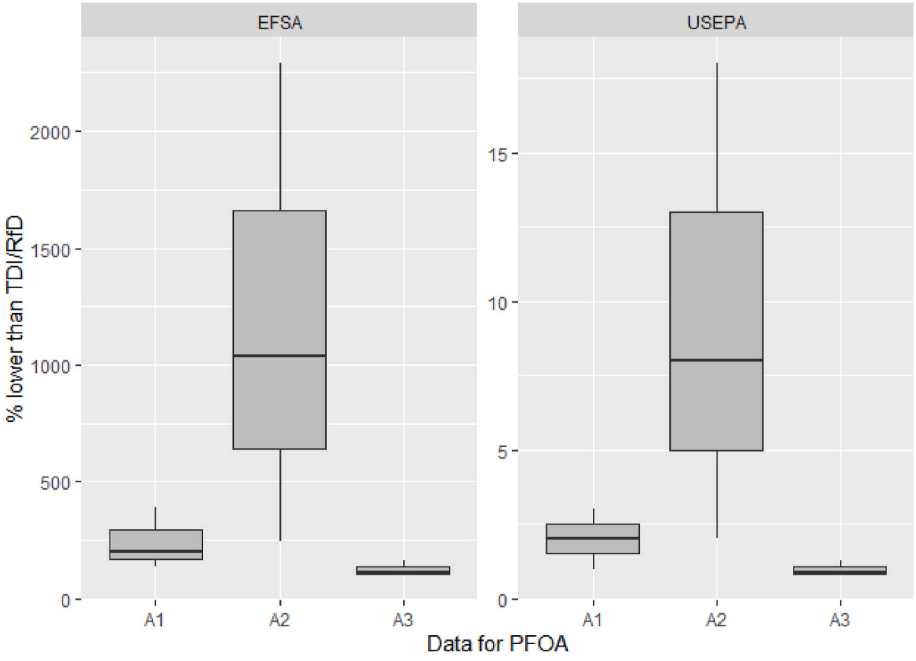

Fig. 3. Comparison with the TDI and RfD sets by regulatory bodies for PFOS. A1 and A2 represent the daily exposure simulated in1-year-old children formula-fed (n = 21) and breastfed (n = 80) respectively (Abraham et al., 2020). A3 represents the daily exposure simulated by PBPK in 5-year-old children (Grandjean et al., 2012).

scaling for chemicals such as PFAS, which are not metabolized? Also, tubular secretion and high resorption of chemicals can affect scaling. Therefore, explaining chemicals with renal excretion is not amenable to allometric scaling (Nair and Jacob, 2016). Thus, limited data and variation in clearance rates reported by several authors make difficult to validate such scaling.

6. Discussion for improving risk assessment

Throughout the world, regulatory and public agencies have been setting limits for persistent chemicals or groups of chemicals such as PFAS with the objective of preventing human health and environmental risks. Over the past 20 years, a variety of health effects related to PFAS exposure have been reported. These range from decreased birth weight, adverse reproductive effects, liver toxicity, developmental toxicity, increase in cholesterol levels, altered immune and thyroid function, and cancer (Knutsen et al., 2018; Alexander et al., 2008). With new scientific strategies, including epidemiological studies, effects in animals, toxicokinetic, and observed levels in human blood, PFAS safe limits have been decreased over time from micrograms to nanograms. There are still

Table 2
Animal to Human Equivalent Doses (HED) based on BSA (Adapted from USFDA).

Species	Divide animal dose by	Multiply animal dose by
Mouse	12.3	0.08
Hamster	7.4	0.13
Rat	6.2	0.16
Ferret	5.3	0.19
Guinea pig	4.6	0.22
Rabbit	3.1	0.32
Dog	1.8	0.54
Monkeys (cynomolgus, rhesus, and stumptail)	3.1	0.32
Marmoset	6.2	0.16
Squirrel Monkey	5.3	0.19
Baboon	1.8	0.54
Micro-pig	1.4	0.73
Mini-pig	1.1	0.95

fundamental gaps in the way limits that are being established using specific data and processes. Based on our review, we emphasize some of the shortcomings in existing research and introduce an improved framework for assessing risk.

The first question is about using a conventional approach to assess the toxicity of individual chemicals (PFOS, PFOA, etc.) when in fact, human beings are exposed to a complex mixture of PFAS. It is well known that co-exposure to chemicals, especially different types of PFAS may be synergistic or antagonistic. Discussions on whether PFAS should be addressed using an additive approach, relative potency framework, or toxic equivalency factor approach are ongoing. Substances could be grouped by their persistence (toxicokinetic), bioaccumulation, biological functions, or molecular initiating events, with potency factors derived from several assays, or subclassifications (structural similarity). Also, the mechanisms behind the combined effect of mixtures of PFAS have not yet been elucidated. A recent study reported that individual and binary combinations of PFAS (PFOS, PFOA, PFNA, PFHxS, and PFDA) have concentration-dependent cytotoxicity and decreased GSH levels in HepG2 cells (Ojo et al., 2021). Developing a grouping strategy to evaluate the potential health effects of a large number of PFAS, requires consideration of PFAS heterogeneity. In recent years, subclass names have been proposed (Sha et al., 2019; Wang et al., 2017). However, there are still major disagreements regarding these groupings. For example, exposure routes may be complex and biological persistence and elimination half-lives are still not predictable using in silico tools based on structure. For a large number of PFAS, traditional approaches to evaluating toxicity may not be feasible. New approach methodologies (NAM), which include a combination of in vitro high-throughput toxicity screening and in silico models, are necessary to inform further testing of PFAS (Patlewicz G., 2020).

Secondly, different population groups can have different risk profiles for these chemicals. There have been multiple pieces of evidence that point towards a higher concentration of PFAS in children and older-age populations. The C8 health project study conducted in 69,030 U.S. participants who were enrolled over 13 months (2005–2006), showed lower median PFAS (PFOS, PFOA, PFHxS, and PFNA) concentrations in women than in men. Also, the reported concentrations of PFOS and PFOA were relatively lower in adults (20–39 years of age) than in teenagers (12–19 years of age) and people over the age of 60 years (Frisbee et al., 2009). Additionally, a great amount of uncertainty and variability in published data often makes difficult to reach clear conclusions. In most epidemiological studies, the variability is very high, which leads to inconsistent results.

Third, reporting of methodological protocols and results of epidemiological and animal studies must be consistent and follow a standardized protocol set by several regulatory bodies. Statistical bias, consideration of confounders, and multiple sampling for human biomonitoring over a specified time period, are required to increase the confidence level in the research findings. Inclusion of types and numbers of animals, computerized randomization, and descriptions of deviations from the protocol in published articles, will reduce bias in and help strengthen the evidence from animal studies. When deciding dosing levels for animals, a human-relevant dose needs to be appropriately calculated in order to further link associations with human risk assessment and help set TDI for humans. Also, sometimes uncertainty in studies with different in-vivo models and epidemiology is so high especially for endpoints like immunotoxicity that it becomes difficult to translate the results for risk assessment (Vidal et al., 2021, in press). In such cases, utilizing different computational models help in filling some gaps and improving translation of risk.

Advancements in computational toxicology and availability of experimental data is beginning to shift the focus of research on PFAS towards translation of risk from exposure scenarios to health risk assessment using in-silico tools. Translational toxicology involves the integration of pharmacokinetics, pharmacodynamics, systems biology, and adverse outcome pathways (AOPs) in order to understand the interactions among chemicals and living beings at different levels of biological organization (Kumar et al., 2020). Compared to traditional dose-response models, integrative translation toxicology implements a more complex structure, as shown in Fig. 4. In-silico tools such as PBPK models, are currently being utilized to estimate daily exposure based on serum levels found in the population, together with additional evidence from epidemiology and animal studies. A large amount of data is required for translation toxicology which often becomes the limiting factor but, in such cases, semi-mechanistic model can be used. In-silico techniques including QSAR, PBPK, and read-across, help to reduce the need for experimental studies and to provide strong evidence for toxicity assessment and grouping of persistent chemicals (Deepika et al., 2020). The translational framework should combine different approaches such as in-silico, in-vitro, in-vivo, epidemiological studies, and omics techniques, for integrating results and characterizing biological and toxicological risks from xenobiotic exposure. Such kind of translational framework can help to fill some of the existing gaps, including incorporating sensitivity populations, reducing uncertainty in interspecies extrapolation, and identifying mechanisms. With more than 150 PFAS (long-chain and short-chain) currently registered, it is difficult to group them and evaluate individual and co-exposure toxicities based on current experimental and epidemiological data. A detail discussion on PFAS co-exposure and limitations of the conventional approach has been discussed in the report of this project (Deepika et al., 2022). The translational framework is helpful in these cases, especially for chemical hazard characterization by grouping chemicals and characterizing potency, based on in-silico and experimental data.

7. Conclusions and future actions

Like other harmful and potentially harmful chemicals, many PFAS have potential to produce a wide range of adverse health effects. Establishing health effects guidelines requires a careful review of the strength of evidence, consistency of evidence across studies, species concordance, strength of effect associations in epidemiological studies, and selection of effect/s for determining which potential impacts are either severe, or of great concern.

Two of the reviewed studies found an association between PFAS exposure and negative immune outcomes, but some inconsistency has been observed. Although recent studies have associated exposure to PFAS with adverse health outcomes, most are cross-sectional analyses. Therefore, data are insufficient to draw accurate conclusions about the association of PFAS with any specific disease. With some recent evidence of selected PFAS involvement in immune hazards to humans, future human studies must characterize wider immune outcomes including (but not limited to) immune effects from early exposure during pregnancy and the possible role of PFAS's in initiating allergic and autoimmune processes, conditions for which a dose-response is hard to predict.

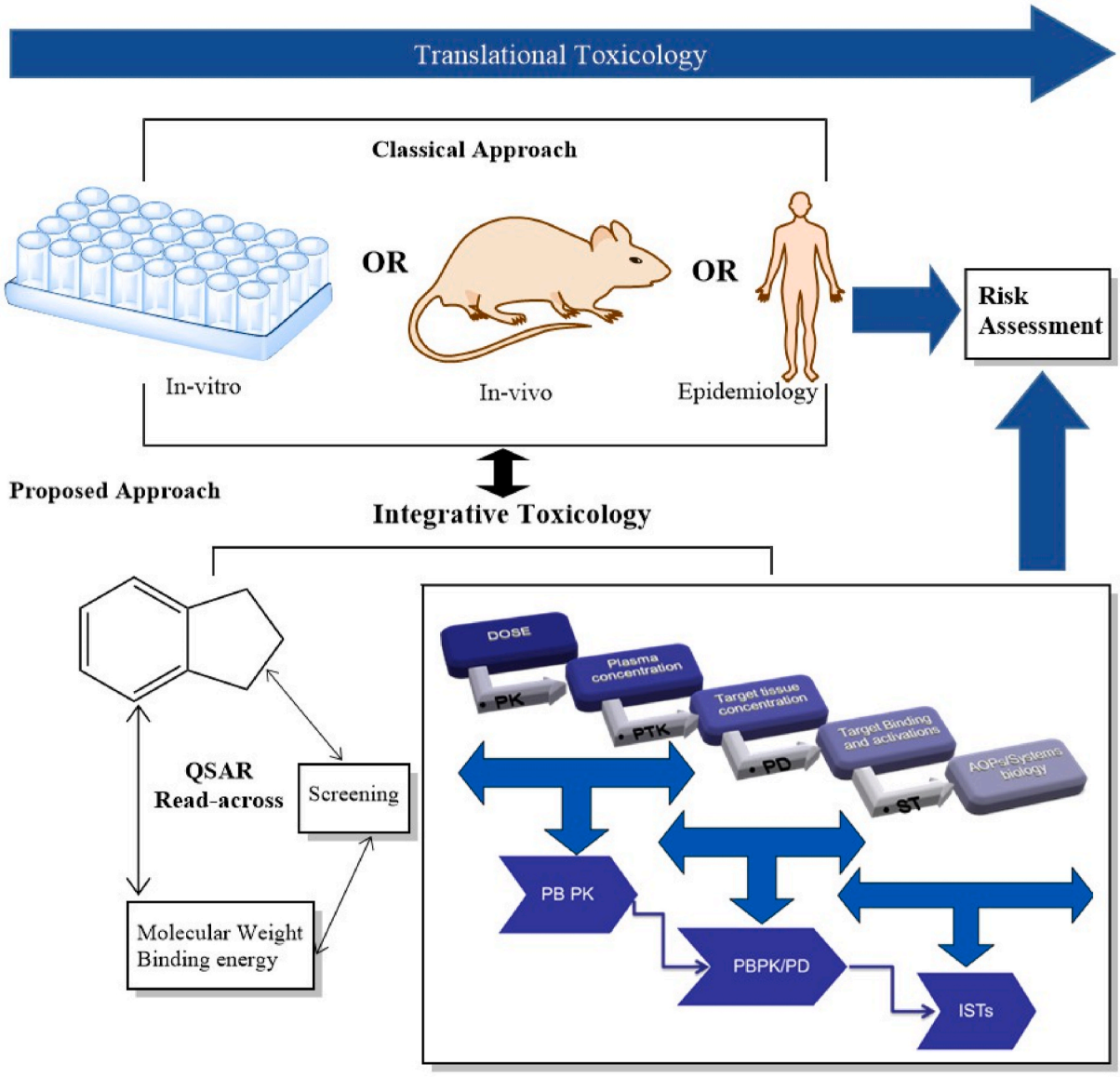

Fig. 4. Proposed framework for integrational toxicology. Integrative toxicology includes incorporating both experimental studies like in-vitro, in-vivo, or epidemiological and in-silico models like PBPK, PD, QSAR, and read-across to improve human health risk assessment (Sharma, 2018).

More longitudinal epidemiology studies are required with additional susceptible human endpoints. In particular, prospective vaccination studies covering more varied types of vaccines and including different populations, as well as additional studies on other human immune outcomes. Epidemiological studies must also include cumulative exposures of several PFAS. Particularly, studies of the effects of PFNA and PFHxS on the immune system should be conducted.

Significant toxicokinetic differences have been observed in various animal and human studies. For example, in rodents, half-life varies from a few hours to several weeks, being in general much shorter than that in humans. For some PFAS's, interactions with various transporters involved in the reabsorption processes, occurring at the hepatic, intestinal and renal levels have been observed, which affects the elimination half-life. The half-life of PFAS has been a major controversial issue, with varying PFAS half-lives in human differing by years.

Although PBPK models for some PFAS are well developed for health risk assessment, they still need to be further developed, optimized, and validated for other PFAS. Experimental evidence is needed to understand and quantify the association between PFAS and blood lipids (cholesterol levels) and the role of enterohepatic recirculation and glomerular reabsorption, including organic anion transporters.

In the case of mixture toxicity - "an additive RfD approach" compared to a "relative potency factor (RPF) approach," should be considered. However, for most PFAS, RPF values are still lacking for susceptible hazard endpoints. The development of new approaches to determining PFAS toxicity must consider tissue-specific modes of action. New approaches should rely on molecular interactions involving enzymes, storage, transport proteins, and ability to alter cell membrane fluidity within a particular organ/system. Collaborative development of new AOPs should be encouraged. However, predictive results should be biologically plausible and represent dose-effect response for human. A greater emphasis on developing workable and effective risk assessment methods for human health, including AOPs to support regulatory processes and development of relevant policy-related strategies are clearly necessary.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2022.112722.

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